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DEVELOPMENTAL TOXICOLOGY OF METHYLPHOSPHONIC DIFLUORIDE (DF)

by William C. Starke
David C. Burnett
Roger J. Hilaski
James H. Manthei
Dale H. Heitkamp
Stanley P. Liebenberg, LTC, V.C.
Dean M. Bona
Robert D. Moore

RESEARCH DIRECTORATE

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The teratogenicity of vaporized methylphosphonic difluoride (DF) was investigated using the rat and rabbit as the experimental models. Pregnant rats and rabbits were exposed to chamber concentrations of 0.2, 1.0, and 10.0 mg/m^3, 6 hr/day for 10 and 13 days, respectively. Dams and does exposed at the 10.0 mg/m^3 level exhibited nasal exudate and raspy breathing. These dams also had significantly lower body weights on the last three of four weighings. There were, however, no differences between the control litters and the DF-exposed litters for the parameters: litter number, mean body weight, mean crown-rump length, visceral or skeletal malformations. It was concluded that DF is not teratogenic in the rat or rabbit by the inhalation route at the concentrations tested.

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Hilaski, Roger J.; Manthei, James H.; Heitkamp, Dale H.;
Liebenberg, Stanley P., LTC, V.C.; Bona, Dean M.; and Moore, Robert D.

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PREFACE

The work described in this report was authorized under Project Number 1L162622A554, Retaliatory Chemical Munitions. This work was started in October 1985 and completed in April 1986. The experimental data are contained in laboratory notebooks 85-0181, 85-0204, 85-0216, 85-0221, 85-0222, and 86-0055.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," National Institute of Health Publication No. 85-23. These investigations were also performed in accordance with the requirements of AR 70-18, Laboratory Animals, Procurement, Transportation, Use, Care, and Public Affairs.

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DEVELOPMENTAL TOXICOLOGY OF METHYLPHOSPHONIC DIFLUORIDE (DF)

1. INTRODUCTION

Health hazard testing of methylphosphonic difluoride (DF) is needed to establish permissible exposure limits for those who handle or work around this material. The literature contained no information on teratogenic effects.

2. MATERIALS

DF is in a group of compounds whose effects on reproduction have not been extensively investigated at this Center. Work conducted by this Center with other organophosphates, i.e., dimethyl- and diethyl-morpholinophosphoramide (DMMPA, DEMPA), showed no effect on reproduction;[1] however, work by Dunnick,[2] with dimethylmethylphosphonate (DMMP), showed effects on spermatogenesis and embryonic survival. With two differing responses to organosphosphates, it is obvious that one cannot project the effects of such compounds based on composition.

The compound is relatively volatile and extremely reactive. It hydrolyzes very readily to methylphosphonic fluoride (MF) and hydrogen fluoride (HF). The expected route for any human exposure would be by inhalation. However, the potential exists for dermal, ocular, and oral exposures. Hydrolysis would occur when the vapors came into contact with mucous membranes. The exposures for these studies were whole body exposures in an atmosphere of vaporized DF. The major route of exposures was by inhalation. The exposures were conducted in dynamic flow chambers (at 15 air changes/hr, 750 L/min). Positive control chemicals were given by intraperitoneal (ip) injections.

2.1 Chemicals.

The following chemicals were used in exposure procedures: DF, with a purity of 98% by NMR, was used for inhalation exposures; ethylenethiourea (ETU), at a concentration of 30 mg/mL for a dose level of 240 mg/kg, was used as a positive control for rats; and 6-aminonicotinamide (6-AN), at a concentration of 100 mg/mL in 10% aqueous solution of gum acacia for a dose of 2.5 mg/kg, was used as a positive control for rabbits.

2.2 Animals.

Rats and rabbits were the animals of choice for this work. Sprague-Dawley rats from Charles River were used. Two hundred, 10-wk-old, females and one hundred, 8-wk-old, males were received for mating. Seventy-two timed pregnant female New Zealand rabbits were received from Hazelton Research Products, Incorporated, Denver, PA. Excess animals were ordered to allow for unexpected deaths or unsuitability of the animals because of illness or injury.

The rabbits had been bred in small groups on five successive days for ease of handling on derivation days. The use of rabbits is in accordance with Food and Drug Administration (FDA) regulations.[3]

2.3 Housing.

Until the day before the first day of their exposure periods, the animals were housed in holding rooms adjacent to the chamber area. These rooms were maintained at a temperature of 75 $\pm$ 5 °F with a relative humidity of 40-60%. Rats and rabbits were held in separate rooms. Lighting was automatically controlled for 12-hr light/12-hr dark. The rats were singularly caged in stainless steel hanging cages measuring 10 in. long by 7 in. wide by 7 in. high. The rabbits were singularly caged in stainless steel cages which measured 30 in. long by 22 in. wide by 24 in. high. Waste pans beneath the cages were lined with absorbent paper that was changed every other day. Food was available in hanging cups for both rats and rabbits; water was available for both sets of animals through automatic watering nipples. Water was provided in hanging cups if it was not possible to use the automatic nipples.

The species were exposed at separate periods. During their exposure periods, the animals were housed in rooms adjoining the chamber rooms. The positive and negative control animals were kept in a separate room from the animals being exposed to DF. At the end of each day's exposure, the animals were returned to these rooms. At the conclusion of the total exposure period, the animals were returned to their original holding rooms where they were maintained until their scheduled necropsy dates.

2.4 Exposure Chambers and Cages.

Animal exposures were conducted in four walled, stainless steel 3000-L chambers equipped with wire-reinforced, glass-paneled doors. The chambers measured 59.5 x 59.5 x 51.5 in. The DF, contained in a syringe, was injected into a plenum where it was vaporized and carried into the chamber on a stream of purified air at a flow rate of 750 L/min. The chambers' concentrations were monitored using a HyFED phosphorous analyzer [Model PA260 (Columbia Scientific Industries Corporation, Austin, TX)] equipped with a flame photometric detector (FPD). The airflow provided the appropriate negative air pressure within the chamber to preclude the release of DF into the room. The chamber concentrations were monitored repeatedly during the 6-hr exposure periods. The target concentrations were 0.1, 1.0, and 10.0 mg/m^3. However, the lowest actual concentration reached was 0.2 mg/m^3. The daily average chamber concentrations are presented in Tables 1 and 2. The pregnant animals were exposed throughout the organogenesis period of their pregnancies. For the rat, this period was from day 6 to day 15 of gestation; for the rabbit, it was from day 6 to day 18 of gestation.

Table 1. Average Daily Chamber Concentrations--Rat Teratology

Dose level	Low (0.2 mg/m³)	Medium (1.0 mg/m³)	High (10.0 mg/m³)
	Target level		
Average daily concentration			
1	0.20 $\pm$ 0.02	1.00 $\pm$ 0.05	10.32 $\pm$ 0.37
2	0.21 $\pm$ 0.01	1.01 $\pm$ 0.02	10.00 $\pm$ 0.23
3	0.21 $\pm$ 0.01	1.01 $\pm$ 0.03	11.28 $\pm$ 2.86
4	0.21 $\pm$ 0.01	1.02 $\pm$ 0.04	9.74 $\pm$ 0.41
5	0.21 $\pm$ 0.01	1.03 $\pm$ 0.04	10.55 $\pm$ 0.53
6	0.21 $\pm$ 0.00	1.03 $\pm$ 0.01	10.10 $\pm$ 0.21
7	0.22 $\pm$ 0.01	1.02 $\pm$ 1.19	9.66 $\pm$ 1.19
8	0.21 $\pm$ 0.01	0.99 $\pm$ 0.05	9.70 $\pm$ 0.21
9	0.20 $\pm$ 0.01	1.01 $\pm$ 0.04	10.00 $\pm$ 0.18
10	0.21 $\pm$ 0.02	1.05 $\pm$ 0.08	9.91 $\pm$ 0.66
11	0.21 $\pm$ 0.02	1.02 $\pm$ 0.04	10.03 $\pm$ 0.54
12	0.21 $\pm$ 0.01	1.03 $\pm$ 0.05	9.90 $\pm$ 0.28
13	0.21 $\pm$ 0.01	1.01 $\pm$ 0.01	9.77 $\pm$ 0.29
Average total concentration	0.209 $\pm$ 0.014	1.02 $\pm$ 0.041	9.87 $\pm$ 1.73

Table 2. Average Daily Chamber Concentrations--
Rabbit Teratology

Dose level	Target level Low (0.2 mg/m^3)	 Medium (1.0 mg/m^3)	 High (10.0 mg/m^3)
Average daily concentration			
1	0.20 ± 0.00	1.04 ± 0.02	9.80 ± 0.40
2	0.20 ± 0.00	1.00 ± 0.06	14.65 ± 0.98
3	0.21 ± 0.01	1.14 ± 0.12	14.90 ± 0.56
4	0.21 ± 0.01	1.09 ± 0.07	14.60 ± 0.30
5	0.21 ± 0.01	1.05 ± 0.04	9.70 ± 0.21
6	0.20 ± 0.01	1.03 ± 0.03	10.20 ± 0.60
7	0.21 ± 0.01	1.05 ± 0.04	10.45 ± 0.76
8	0.21 ± 0.01	1.03 ± 0.05	9.90 ± 0.60
9	0.20 ± 0.01	1.07 ± 0.05	10.30 ± 0.30
10	0.21 ± 0.01	1.12 ± 0.10	10.00 ± 0.25
11	0.21 ± 0.20	0.93 ± 0.25	9.97 ± 0.05
12	0.20 ± 0.01	1.00 ± 0.02	10.16 ± 0.30
13	0.20 ± 0.01	1.00 ± 0.03	10.55 ± 0.45
14	0.21 ± 0.02	1.02 ± 0.02	10.40 ± 0.48
15	0.20 ± 0.01	1.04 ± 0.03	10.32 ± 0.40
16	0.20 ± 0.02	1.05 ± 0.02	10.03 ± 0.24
Average total concentration	0.205 ± 0.012	1.041 ± 0.089	10.68 ± 2.04

2.5 Caging for Exposure.

During exposure, all animals were placed in compartmentalized, stainless steel cages without food, water, or bedding. The compartments measured 10 in. long by 5 in. wide by 5 in. high with 10 compartments per cage for the rats. For the rabbits, the compartments were 30 in. long by 25 in. wide by 22 in. high with 4 compartments per cage. These size cages insured enough room for comfort and position changes.

3. METHODS

3.1 Rabbits.

Timed pregnant rabbits were received from the supplier on a staggered basis. Upon receipt, the does were randomly assigned across dose groups. The exposure period for the rabbits began on the calculated 6th day of gestation and continued 6 hr/day for 13 consecutive days. Positive controls were administered 2.5 mg/kg of 6-AN ip on day 13 of gestation.

On day 30 of gestation, the does were euthanized using ear vein injections of the euthanasia fluid, T-61. Their fetuses were immediately delivered by caesarean section. The total number of implantation sites in each uterine horn, the weight of the intact uterus, the number of viable fetuses, and the number of late deaths or resorptions were recorded. The fetuses were removed from the horns, and each fetus was examined grossly for external abnormalities, sexed, measured (crown-rump), and weighed.

Viable fetuses were then euthanized by CO_2 using foil-covered polycarbonate rat cages as euthanizing chambers. The fetuses were decapitated; the heads and bodies were co-numbered. The heads were placed in Bouin's solution for serial sectioning at a later date. A fresh autopsy was conducted on each fetal body, and visceral abnormalities were characterized and recorded. The bodies were placed in 95% EtOH and, after several days, were placed in 2% KOH with alizarin red stain for digestion of the muscle and staining of bone.

Gradually, the bodies were changed over to a 70% v/v glycerin/water solution, using a crystal of thymol as an antifungal agent. The heads were serially sliced and evaluated according to Wilson's method.[4] The stained bodies were examined for skeletal abnormalities. The fresh autopsy/stained skeletal examination is a method proposed by Robert Staples of the Haskel Laboratories [Dover, DE (personal communication)].

3.2 Rats.

The rats were selected for mating on a random basis. On the afternoon prior to the first night of mating, each male was placed in a polycarbonate cage. At the end of the workday, two

randomly selected females were placed in the cages with each of the males. Water and food were available to the animals at all times. The females were checked for sperm each morning by performing a saline wash of their vaginas and microscopically examining this vaginal wash. The day on which sperm were found in the washings was considered day zero of gestation.[5] On the day that it was considered to be sperm positive, a female was assigned to one of the control groups or to one of the exposure groups in such a fashion that, as near as possible, an equal number of pregnant animals were assigned to each group each day. If it was not sperm positive, a female was returned to its own home cage for the day and returned to a male's cage that evening. This format was repeated until each group was assigned 24 sperm-positive dams.

The exposure period for the inseminated females began on the calculated 6th day of gestation and continued 6 hr/day for 10 consecutive days. Positive controls were administered 240 mg/kg of ETU by gavage on day 9 of gestation. On day 20 of gestation, the rats in each exposure group were euthanized in CO_2 chambers, and the fetuses were immediately delivered by caesarean section. The total number of implantation sites in each uterine horn, the number of viable fetuses, and the number of resorptions were recorded. Upon removal from the uterus, each fetus was examined grossly for external abnormalities, sexed, measured (crown-rump), and weighed. Half of each litter (usually even numbered) was placed in Bouin's fixative and later examined for visceral abnormalities using Wilson's serial sectioning technique.[4] The other half of the fetuses was placed in 95% ethanol (EtOH) and was later eviscerated, cleaned in 1% potassium hydroxide (KOH) stained with alizarin red in Mall's Solution, and transferred to glycerin for evaluation of the skeletal system.[6] The experimental design appears in Table 3.

4. RESULTS

4.1 Rats.

The mean body weights for dams in the 10-mg/m^3 dose groups were significantly lower than the weights for control dams on gestation days 6, 15, and 20 (Table 4). Dams exposed to DF at the 10-mg/kg level showed raspy breathing and nasal exudate. However, the mean fetal body weights did not differ significantly when any of the litters from the DF-dosed dams were compared with those from the negative control group. Using analysis of variance (ANOVA), it was shown that for fetal weights and crown-rump lengths, there was no significant lowering of weight or body length associated with maternal exposure to DF vapor during organogenesis. However, fetuses from dams treated with 240 mg/kg of ETU showed a significant lowering of body weight and crown-rump length when compared with the negative controls (Tables 5 and 6). Significance was determined using Student's t-test or ANOVA.

Table 3. Experimental Designs for Rabbits and Rats

Condition	Exposure duration (hr)	Number pregnant	Exposure period (days)	Comments
Rabbits				
(-)Control*	6	12	6 to 18	Does euthanized on day 30 of gestation. Fetuses examined for visceral and skeletal abnormalities.
0.1 mg/m DF	6	12	6 to 18	
1.0 mg/m DF	6	12	6 to 18	
10.0 mg/m DF	6	12	6 to 18	
(+)Control**	--	12	9	Single ip dose
Rats				
(-)Control*	6	20	6 to 15	Dams euthanized on day 20 of gestation. Fetuses examined for visceral and skeletal abnormalities.
0.1 mg/m	6	20	6 to 15	
1.0 mg/m	6	20	6 to 15	
10.0 mg/m DF	6	20	6 to 15	
(+)Control†	6	20	11	Single oral dose

*Negative control--0.0 mg/m DF

**Positive control--2.5 mg/kg 6-aminonicotinamide

†Positive control--240 mg/kg ethylene thiourea (ETU)

Table 4. T-Test of Weight Gain in Pregnant Rats from First to Fourth Weighings During Exposure to DF or ETU

Dose group	International mean	Mean gain	T value*
0.0 mg/m	277.4	101.3	1.34
0.2 mg/m	275.7	91.5	--
0.0 mg/m	277.4	101.3	1.90
1.0 mg/m	277.2	80.6	--
0.0 mg/m	277.4	101.3	5.02**
10.0 mg/m	274.0	45.4	--
0.0 mg/m	277.4	101.3	-0.16
6 AN	257.3	103.7	--
0.2 mg/m	275.7	91.5	1.00
1.0 mg/m	277.2	80.6	--
0.2 mg/m	275.7	91.5	4.14**
10.0 mg/m	274.0	45.4	--
0.2 mg/m	275.7	91.5	-0.82
6 AN	257.3	103.7	--
1.0 mg/m	277.2	80.6	2.55**
10.0 mg/m	274.0	45.4	--
1.0 mg/m	277.2	80.6	-0.83
6 AN	257.3	103.7	--
10.0 mg/m	274.0	45.4	-2.05
6 AN	257.3	103.7	--

*At 26 degrees of freedom

**Significant at 95% confidence

Table 5. Effect of Maternal Exposure to DF During Organogenesis on Mean Fetal Weights in Rats

Dose group	Mean weight	Standard deviation	Sample size
(-)Control*	3.5	0.4	363
0.2 m	3.5	0.4	349
1.0 m	3.5	0.3	305
10.0 m	3.5	0.4	292
(+)Control**	2.4	0.3	284
Dose group	**Mean crown-rump length**	**Standard deviation**	**Sample size**
(-)Control*	3.5	0.3	363
0.2 m	3.4	0.3	349
1.0 m	3.4	0.3	305
10.0 m	3.5	0.2	292
(+)Control**	2.6	0.3	284

*Negative control--room air

**Positive control--340 mg/kg ETU

Table 6. BMDP7D Difluoro Caesarean Section and Delivery Data

```
              ************                                                                  ************
Histogram of  * CR       * (Variable 6).  Cases Divided into Groups Based on Values of      * Dose     * (Variable)
              ************                                                                  ************

                              0.2 mg/m3          1.0 mg/m3          10 mg/m3           ETU
           Control            Low                Medium             High               PCONTROL
                            +                  +                  +                  +                  +
Midpoints
   4.240)**
   4.160)
   4.080)*
   4.000)*****************  *********          ****************   **********
   3.920)***                *                  *                  *****
   3.840)***************25  *************      ****************   *************
   3.760)
   3.680)***************34  ***************28  ***************22  *************
   3.600)***************45  ***************26  ***************24  ***************20
   3.520)***************92  ***************94  ***************83  **************120 ***
   3.440)M**************45  M**************40  M**************42  ***************35 *
   3.360)
   3.280)***************36  ***************50  ***************33  ***************25 *
   3.200)***************24  ***************42  ***************24  ***************26 **
   3.120)******             ************       ***************    ***                *
   3.040)**************     ***************21  *****************  *************      ****************74
   2.960)
   2.880)*****              **                 ****               *                  ****
   2.800)***                **                                    *                  ****************21
   2.720)***                *                  *                                     ****************28
   2.640)*                  *                                                        M***************21
   2.560)
   2.480)*                  *                                                        ****************53
   2.400)*                                                                           ****************26
   2.320)                                                                            ****************21
   2.240)                   *                                                        *********
   2.160)
   2.080)                                                                            *****
   2.000)                                                                            *********
   1.920)                                                                            *
```

Group means are denoted by M's if they coincide with *'s, N's otherwise

Fetuses from rats dosed with ETU had major and, usually, multiple defects at a significantly higher frequency than fetuses from any other group. The visceral abnormalities included: harelip, fused olfactory buds, distorted olfactory buds, undeveloped lens, underdeveloped retina, folded retina, cleft palate, exencephaly, hydrocephaly, enlarged thymus, right aorta, right ductus arteriosus, enlarged liver, small stomach, extruding intestines, dilated kidneys hydroureter, underdeveloped kidneys, large bladder, underdeveloped bladder, underdeveloped gonads, and undescended gonads. Skeletal abnormalities included: deformed cranium, mandible and maxilla; deformed vertebra; deformed long bones; deformed scapula and clavicle; deformed ribs; rudimentary ribs; fused ribs; and extra and wavy ribs. In the 0.2- and 10.0-mg/m^3 DF groups, there were twice as many fetuses that exhibited dilated lateral ventricles than among the control fetuses. Ossification of nasal, sternal, and metacarpal bones, and instances of wavy ribs were increased in the DF-exposed groups over the control group. There was generalized retarded ossification of bone in these fetuses. These results are shown in Tables 7 and 8.

4.2 Rabbits.

The mean body weights of does, over the four weighing periods, showed no adverse response to DF exposure (Table 9). Does exposed to DF at the 10.0-mg/kg level exhibited heavy nasal exudate and tearing; moist nares were evident up to 8 days beyond the last exposure. Three does in the 1.0-mg/m^3 group had very low food consumption for 10 days. Two of these does were pregnant and their litter weights were also low. The mean litter size for the control group was 8.0 fetuses; the mean for the 1.0 mg/m^3 was 10.8 and for the 10.0-mg/m^3 group, 9.5. ANOVA and Tukey's test showed a significant difference in the fetal weights, and the histogram showed the mean fetal weights for all DF-exposed groups to be lower than the control mean, with the 1.0- and 10.0-mg/m^3 groups being significantly lower. The crown-rump lengths were commensurate with the weights (Tables 10 and 11). Only one fetus had major abnormalities; that fetus was from a dam in the high-dose DF group. Most of this fetus' viscera were undistinguishable, including the heart, spleen, liver, stomach, gall bladder, adrenals, and gonads (Figure, Tables 12 and 13). There was also one fetus from a dam given 6-AN that did not have a discernible stomach.

Table 7. DF-2 Teratology (Rat)--Number of Fetuses Showing Malformations at Each Dose Level

Visceral malformations	Negative control	Group (mg/m^3) 0.2	1.0	10.0	Positive control
Total fetuses examined	182	175	153	146	132
1. Nasal cavity open	--	--	--	--	123
2. Harelip	--	--	--	--	98
3. Olfactory fused	--	--	--	--	36
4. Olfactory not observed	--	--	--	--	1
5. Olfactory bloody	--	--	1	--	4
6. Olfactory asymmetric	--	--	1	--	23
7. Olfactory has subdermal blood	--	--	--	--	1
8. Lens not observed	--	--	--	--	3
9. Lens underdeveloped	--	1	1	--	13
10. Lens undefined	--	--	--	--	9
11. Retina underdeveloped	--	1	--	--	1
12. Retina folded	--	--	--	--	22
13. Naso-pharyngial open to mouth	--	1	--	--	110
14. Cleft palate	--	--	--	--	110
15. Cranium ruptured	--	--	1	--	142
16. Cranium has subdermal blood	--	--	2	1	38
17. Exencephaly	--	--	1	1	142
18. Brain has subdermal blood	--	--	--	--	7
19. Hydrocephaly	--	--	--	--	60
20. 3rd ventrical undefined	--	--	2	4	29
21. 3rd ventrical dilated	--	--	2	1	74
22. Hydrocephaly in 3rd ventrical	--	--	--	--	12
23. Lats undefined	10	--	9	1	91
24. Lats dilated	7	16	8	15	4
25. Spinal cord has solid core	--	--	1	--	129
26. Spinal cord has subdermal blood	--	--	--	--	4

Table 7. DF-2 Teratology (Rat)--Number of Fetuses Showing Malformations at Each Dose Level (Continued)

Visceral malformations	Negative control	Group (mg/m^3) 0.2	1.0	10.0	Positive control
27. Spinal cord bloody	--	--	--	--	5
28. Thymus large	--	--	--	--	3
29. Aorta on the right	--	1	--	--	1
30. Aorta not observed	14	21	15	3	12
31. Ductus arteriosus right	--	--	--	--	1
32. Ductus arteriosus not seen	12	8	13	5	17
33. Small left atrium	1	1	--	1	5
34. Liver bloody	1	3	3	2	--
35. Liver large	--	--	--	1	3
36. Stomach small	--	--	--	--	123
37. Intestines extruding	--	--	--	--	19
38. Intestines bloody	11	10	9	8	11
39. Kidneys dilated	8	22	5	8	132
40. Kidneys hydroureter	17	21	6	10	99
41. Kidneys underdeveloped	--	--	--	--	10
42. Bladder large	--	--	1	--	4
43. Bladder underdeveloped	--	1	--	--	--
44. Gonads underdeveloped	7	--	3	--	5
45. Gonads undescended	6	7	5	7	54
46. Male	99	94	78	69	71
47. Female	83	73	66	72	77
48. No ductus venosus	6	1	1	1	5

Table 8. DF-2 Teratology (Rat)--Total Fetuses Showing Malformations at Each Dose Level

Skeletal malformations	Negative control	Group (mg/m^3) 0.2	1.0	10.0	Positive control
Total fetuses examined	181	174	152	146	152
1. Nasal deformed	1	7	12	--	141
2. Nasal low ossification	1	3	--	--	41
3. Parietal deformed	--	7	--	--	146
4. Frontal deformed	1	7	--	1	148
5. Frontal low ossification	1	8	--	--	--
6. Mandible deformed	--	7	--	--	146
7. Mandible low ossification	--	8	--	--	1
8. Maxilla deformed	1	7	2	--	141
9. Maxilla low ossification	--	7	--	--	5
10. Interparietal absent	1	--	--	--	72
11. Interparietal low ossification	15	--	--	--	79
12. Supraoccipital absent	1	--	--	--	72
13. Supraoccipital low ossification	18	--	--	--	78
14. Cervical low ossification	--	--	--	--	1
15. Cervical has no ossification	--	--	--	--	24
16. Cervical absent	--	--	--	--	17
17. Thoracic absent	--	--	--	--	8
18. Thoracic low ossification	3	9	4	1	69
19. Thoracic has no ossification	--	2	--	--	66
20. Lumbar absent	--	--	--	--	7
21. Lumbar low ossification	1	7	--	--	128
22. Lumbar deformed	1	--	--	--	2
23. Lumbar no ossification	1	1	--	--	8
24. Sacral absent	--	1	--	--	51
25. Sacral low ossification	2	7	--	--	91
26. Sternum low ossification	31	55	56	46	71
27. Sternum fused	--	6	--	--	96
28. Clavicle low ossification	--	--	--	--	6

Table 8. DF-2 Teratology (Rat)--Total Fetuses Showing Malformations at Each Dose Level (Continued)

Skeletal malformations	Negative control	Group (mg/m^3) 0.2	1.0	10.0	Positive control
29. Clavicle deformed	--	6	--	--	92
30. Clavicle short	--	--	--	--	3
31. Scapula low ossification	--	--	--	--	8
32. Scapula deformed	--	5	--	--	8
33. Scapula short	--	--	--	--	20
34. Humerus low ossification	--	--	--	--	9
35. Humerus short	7	1	--	--	24
36. Radius absent	--	1	--	--	21
37. Radius low ossification	3	7	2	--	113
38. Ulna absent	--	--	--	--	13
39. Ulna low ossification	3	7	2	--	105
40. Metacarpals absent	--	1	--	--	27
41. Metacarpals low ossification	6	18	18	7	64
42. Metacarpals no ossification	--	7	--	--	4
43. Femur low ossification	--	--	1	2	4
44. Femur deformed	--	--	--	--	6
45. Femur short	6	2	3	--	52
46. Tibia absent	--	--	--	--	5
47. Tibia low ossification	--	6	--	2	19
48. Tibia short	6	1	--	--	33
49. Fibula absent	--	--	--	--	6
50. Fibula low ossification	--	3	--	2	9
51. Fibula deformed	--	--	--	--	4
52. Fibula short	6	4	--	--	33
53. Metatarsal low ossification	--	1	--	--	2
54. Metatarsal no ossification	--	--	--	2	7
55. Ilium low ossification	1	--	--	--	10
56. Ilium deformed	--	--	--	--	7
57. Ilium short	1	2	--	--	44
58. Ischium absent	--	--	--	--	10
59. Ischium low ossification	1	6	--	1	107

Table 8. DF-2 Teratology (Rat)--Total Fetuses Showing Malformations at Each Dose Level (Continued)

Skeletal malformations	Negative control	Group (mg/m^3) 0.2	1.0	10.0	Positive control
60. Ischium deformed	--	--	--	--	1
61. Ischium short	2	--	--	2	14
62. Pubis absent	--	1	--	--	33
63. Pubis low ossifica[illegible]	1	7	2	2	107
64. Ribs low ossificatio[illegible]	--	--	--	--	1
65. Ribs short	--	7	--	--	124
66. Ribs deformed	--	--	--	--	19
67. Ribs rudimentary	4	13	12	7	21
68. Extra ribs	--	1	--	--	1
69. Ribs wavy	13	13	20	16	10
70. Ribs fused	--	--	--	--	4
71. Caudal absent	--	2	--	--	112

Table 9. T-Test of Weight Gain in Pregnant Rabbits From First to Fourth Weighings During Exposure to DF or 6-AN

Dose group	International mean	Mean gain	T value*
0.0 mg/m	3.5	0.4	-0.50
0.2 mg/m	3.9	0.6	--
0.0 mg/m	3.5	0.4	1.03
1.0 mg/m	3.8	0.3	--
0.0 mg/m	3.5	0.4	0.91
10.0 mg/m	3.9	0.2	--
0.0 mg/m	3.5	0.4	0.19
6 AN	4.0	0.4	--
0.2 mg/m	3.9	0.6	1.48
1.0 mg/m	3.8	0.3	--
0.2 mg/m	3.9	0.6	1.30
10.0 mg/m	3.9	0.2	--
0.2 mg/m	3.9	0.6	0.68
6 AN	4.0	0.4	--
1.0 mg/m	3.8	0.3	0.19
10.0 mg/m	3.9	0.2	--
10.0 mg/m	3.8	0.3	-0.82
6 AN	4.0	0.4	--
10.0 mg/m	3.9	0.2	-0.75
6 AN	4.0	0.4	--

*At 26 degrees of freedom

Table 10. BMDP7D Difluoro Caesarean Section and Delivery Data for the Rabbit

```
            ************                                                          ************
Histogram of * WT         * (Variable 5).  Cases Divided into Groups Based on Values of * Dose       * (Variable 1)
            ************                                                          ************
```

Midpoints	Control	0.2 mg/m³ Low	1.0 mg/m³ Medium	10 mg/m³ High	ETU PCONTROL
75.000)					
72.500)					
70.000)					
67.500)				*	
65.000)	**	*		**	**
62.500)	****	**		**	***
60.000)	******	******	*	***	***
57.500)	***	*****	***	**	****
55.000)	****	**********	*********	********	****
52.500)	**********	***********	**	***********	****
50.000)	M************	***************	**********	********	***********
47.500)	***********	M********	*********	***********	****
45.000)	*******	******	*****	M**************	M*******
42.500)	******	***************	M********	***********	*********
40.000)	**	****	***********	***********	********
37.500)	****	*****	**************	*******	********
35.000)	*	****	**********	***	*****
32.500)	*	**	***	*****	***
30.000)		*	***		*
27.500)			**	*	**
25.000)			***		*
22.500)			*	***	
20.000)			*		*
17.500)					*
12.500)					

Group means are denoted by M's if they coincide with *'s, N's otherwise

	Control	Low	Medium	High	ETU PCONTROL
Mean	50.049	48.286	41.943	45.946	44.762
Std. Dev.	7.318	7.642	8.794	8.976	10.104
R.E.S.D.	7.158	7.989	9.026	8.713	10.122
S.E.M.	0.851	0.780	0.898	0.880	1.116
Maximum	64.700	65.300	59.300	68.500	65.100
Minimum	33.400	31.100	18.800	22.100	16.400
Sample Size	74	96	96	104	82

```
All Groups Combined *************** Analaysis of Variance Table *****************
(Except cases with  * Test       Source   Sum SQS.   DF  Mean Sq.     F       P
unused values for   *Standard   Between   3419.986    4    855.00   11.45  0.0000
variable dose)      *           Within   33371.783  447     74.66
                    ************* Levene Test for Equal Variances ***************
Mean       46.050 *            Between              4     62.74    2.35  0.0536
Std. Dev.   9.032 *            Within             447     26.72
R.E.S.D     9.118 *********** Tests Not Assuming Equal Variances  *************
S.E.M.      0.425 *Welch       Between              4     12.81   12.70  0.0000
Maximum    68.500 *            Within             219      1.01
Minimum    16.400 *
Sample Size   452 *Brown-      Between              4   3419.99   11.50  0.0000
```

Table 11. BMDP7D Rabbit DF Delivery Data

```
                ************                                                                 ************
Histogram of  * CR         * (Variable 6).  Cases Divided into Groups Based on Values of  * Dose       * (Variable 1)
                ************                                                                 ************

                                    0.2 mg/m3          1.0 mg/m3           10 mg/m3               P
                   Control             Low               Medium               High              PCONTROL
Midpoints                   +                  +                   +                   +                   +
  11.600)                    *                                                          *
  11.400)                                                                               *
  11.200)
  11.000)                    **                 **                                      ***
  10.800)***                 *                                      ***
  10.600)**********          *****              **                  ****                *****
  10.400)****                ****               ****                *                   *
  10.200)***                 ***                *                   ****                *
  10.000)****************    *****************  ************        *****************21 **********
   9.800)M*********          *********          *                   *****               *
   9.600)************        ***************    ******************* ************        *******************
   9.400)*                   M                  ***                 M******             **
   9.200)****                *                  ****                ****                M*
   9.000)*****               ****************   M*************      **********          ************
   8.800)**                                     *****               *****               ***
   8.600)**                  ***********        ********            ********            ******
   8.400)                                       ***                 *                   ****
   8.200)                    *                  **                  **                  *
   8.000)                    *****              **********          ****                ***
   7.800)                                       ***                 *****               **
   7.600)                    ***                ****                *                   **
   7.400)
   7.200)                                       *                   *
   7.000)*                   *                                      *                   **
   6.800)                                       *
   6.600)                                                                               *

Group means are denoted by M's if they coincide with *'s, N's otherwise

Mean          9.781            9.404            9.008            9.337            9.241
Std. Dev.     0.638            0.859            0.862            0.865            0.977
R.E.S.D.      0.607            0.873            0.872            0.891            0.953
S.E.M.        0.074            0.088            0.088            0.085            0.108
Maximum      10.800           11.500           11.000           10.800           11.500
Minimum       7.000            7.000            6.800            7.000            6.500
Sample Size      74               96               96              104               82

All Groups Combined *************** Analaysis of Variance Table *****************
(Except cases with  *  Test       Source     Sum SQS.   DF  Mean Sq.     F       P
unused values for   *Standard   Between      26.145     4    6.54    9.00   0.0000
variable dose)      *           Within      324.705   447    0.73
                    ************* Levene Test for Equal Variances ***************
Mean          9.337 *           Between                 4    0.87    3.34   0.0104
Std. Dev.     0.882 *           Within                447    0.26
R.E.S.D       0.891 *********** Tests Not Assuming Equal Variances **************
S.E.M.        0.041 *Welch      Between                 4   12.24   12.13   0.0000
Maximum      11.500 *           Within                220    1.01
Minimum       6.500 *
Sample Size     452 *Brown-     Between                 4   26.14   19.13   0.0000
```

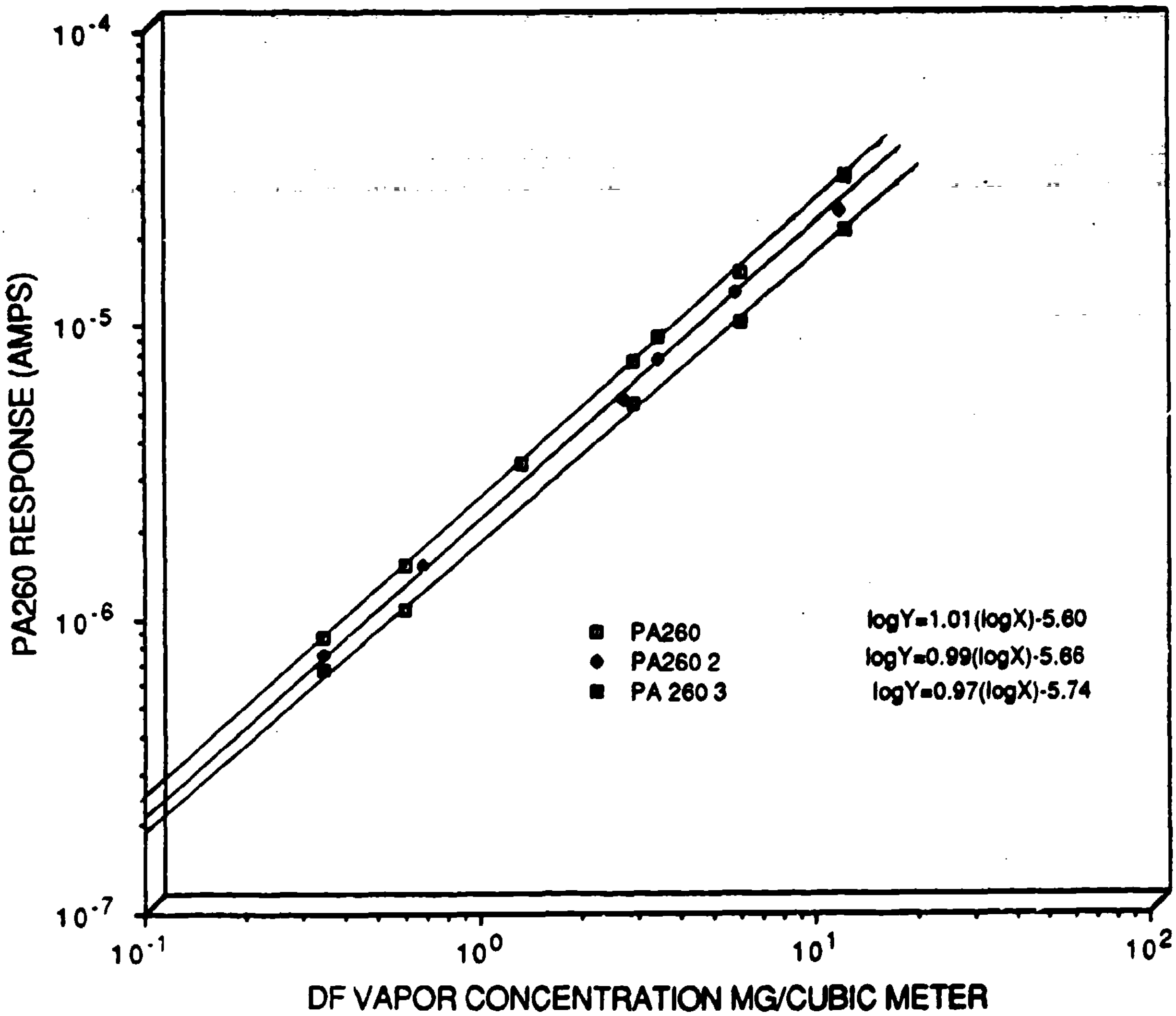

Figure. Calibration Curves for DF Vapor

Table 12. DF-2 Teratology (Rabbit)--Number of Fetuses Showing Malformations at Each Dose Level

Visceral malformations	Dose Level* A	B	C	D	E
Number of fetuses examined	37	48	48	52	41
1. Hole in diaphragm	21	28	39	18	23
2. Bloody abdomen	--	1	--	6	--
3. Bloody paracardial	--	1	--	--	--
4. Fluid in the lungs	--	1	--	--	2
5. Small heart	--	3	--	--	--
6. Heart missing	--	--	--	1**	--
7. Spleen missing	--	1	--	1**	--
8. Liver absent	--	--	--	1**	--
9. Stomach absent	--	--	--	1**	1
10. Gall bladder absent	--	--	--	1**	--
11. Gall bladder large	--	--	--	--	1
12. Thymus small	--	--	--	1	--
13. Adrenals absent	--	--	--	1**	--
14. Enlarged renal pelvis	--	--	--	1	--
15. Kidneys small	--	--	--	2	--
16. Conads missing	--	1	--	1**	--

*Dose Level--A = negative control

B = 0.2 mg/m^3

C = 1.0 mg/m^3

D = 10 mg/m^3

E = positive control

**All in one pup 35949

Table 13. DF-2 Teratology (Rabbit)--Total Fetuses Showing Malformations at Each Dose Level

Skeletal malformations	Negative control	Group (mg/m^3) 0.2	1.0	10.0	Positive control
Total Fetuses Examined	37	48	48	52	41
1. Cervical low ossification	--	--	--	--	2
2. Thoracic low ossification	--	--	--	--	2
3. Lumbar low ossification	--	--	--	--	1
4. Sacral low ossification	--	--	--	--	1
5. Sternum low ossification	40	60	66	59	31
6. Ribs deformed	5	--	--	1	--
7. Ribs rudimentary	18	17	17	15	18
8. Extra ribs	8	13	13	--	5
9. Ribs fused	--	1	--	--	--
10. Caudal absent	3	--	3	--	3

5. DISCUSSION

Work by Crook and co-workers[7] shows the LCt50 for DF in rats to be 295,000 mg-min/m^3. This work also indicated that the irritating properties of DF are so great that a person would readily leave the contaminated area. Any irritation experienced by the dams would have caused stress, and the 10.0 mg/m^3 might explain the lowered maternal body weight. While there were twice as many fetuses in the 0.2- and 10.0-mg/m^3 DF groups exhibiting dilated lateral ventricles than was true for control fetuses, these numbers were only 10% of the number examined in each group--not a significant number. More frequent low bone ossification observed in the DF-exposed group might hint at a retardation of ossification of the sternum and metatarsal bones; such selectivity, however, is not likely.

There were no dental problems in the rabbits, so irritation from the DF might also have been the cause of low food consumption among the DF-exposed does. Low maternal food consumption might have caused the small size of a number of the fetuses, although the increased litter size was the more likely cause. The study reported here showed that forced exposure to the concentration used caused no significant increase in the number of malformations among the fetuses of rats or rabbits. The FDA/EPA Guidelines require that the highest dose used cause some distinguishable sign of toxicity; for this study, the mucus flow from the nares in the rabbit and the raspy breathing and lower weight in the rat were accepted as signs of low toxicity.

The significantly lower body weight of the rabbit fetuses in the 1.0-mg/m^3 group, and the generally lower weight of fetuses in the other groups might, even in the absence of weight loss in the does, indicate a degree of stress as a result of the exposure. However, the highest dose (10.0 mg/m^3) did not result in an increased lowering of fetal weight for that group over the medium dose (1.0 mg/m^3).

6. CONCLUSION

It is evident from the data presented here that DF, at the concentrations used in this study, is not teratogenic or embryocidal in rats or rabbits by the respiratory route.

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